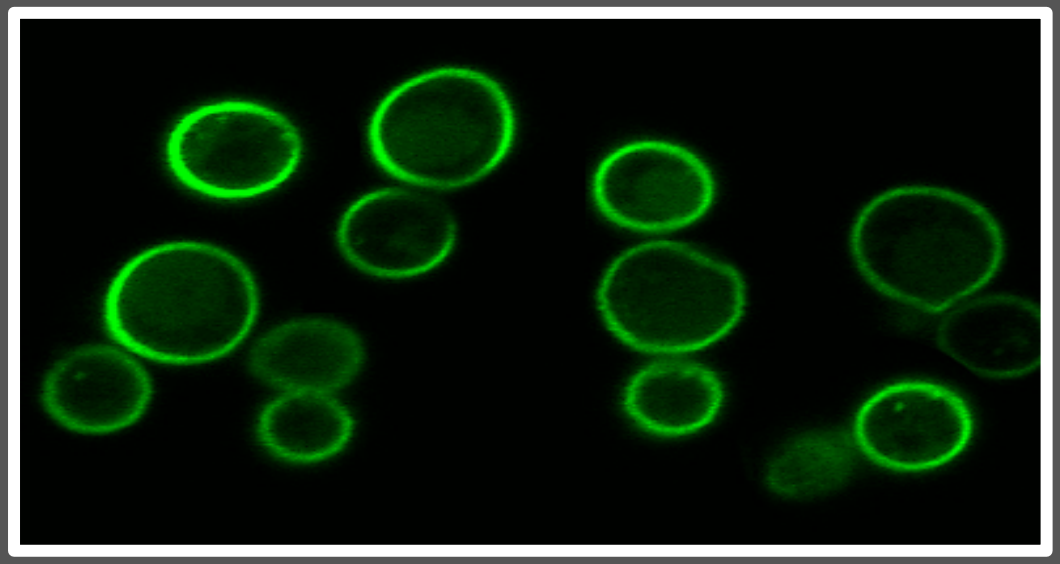
Understanding Parkinson's Disease Pathology in Yeast: A Role for Endocytosis

Maiwase Tembo



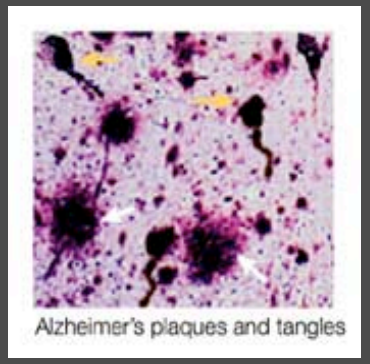
Biology Department, Lake Forest College
Advisor: Dr. Shubhik DebBurman

Road Map

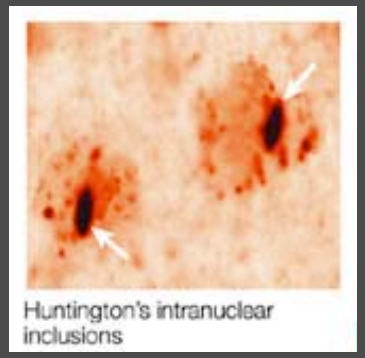
- Introduction
- Hypothesis
- Aims
- Results
- Conclusions
- Future Studies

http://gailbrokaw.files.wordpress.com/2009/02/j0438492.jpg

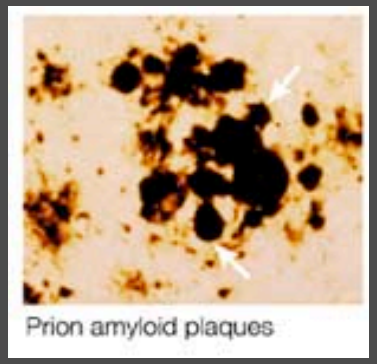
Neurodegenerative Diseases



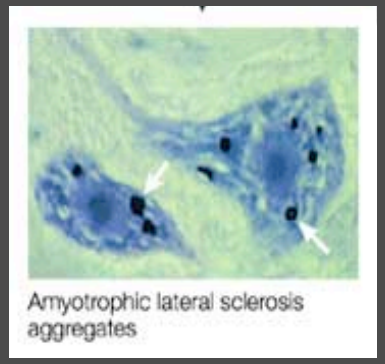
Alzheimer's Disease



Huntington's Disease



Prion Diseases



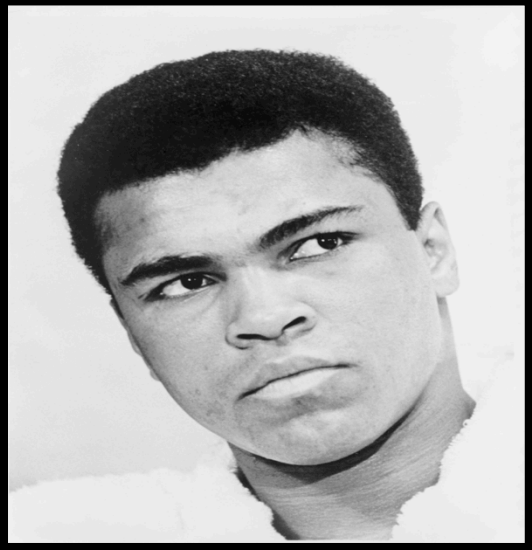
Lou Gehrig's Disease

Parkinson's Disease

Nature Reviews | Neuroscience

Parkinson's Disease

- Hypokinetic movement disorder
- Second most common neurodegenerative disease
- 7 million worldwide
- 1 million in the US
- No known cure



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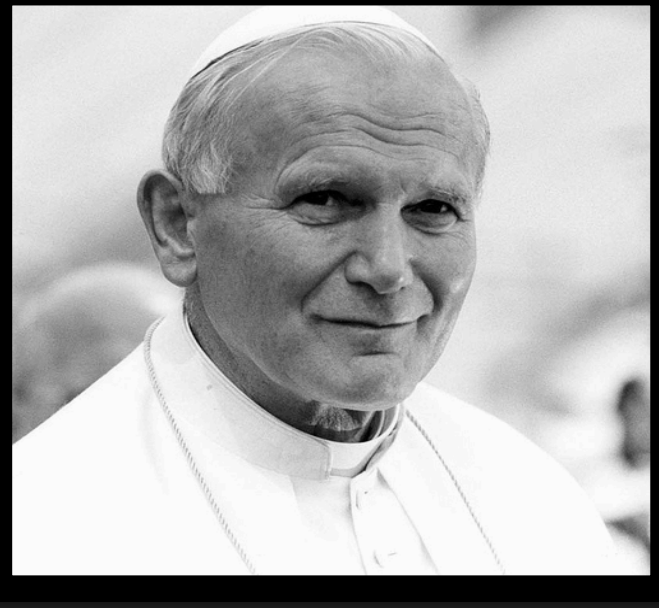

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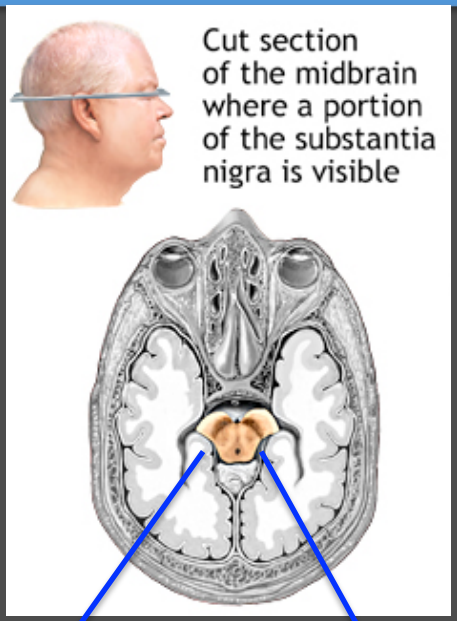
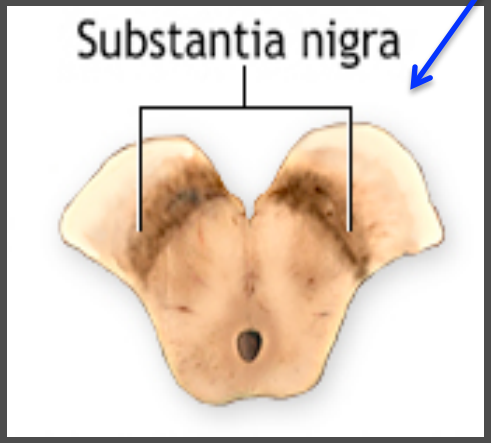
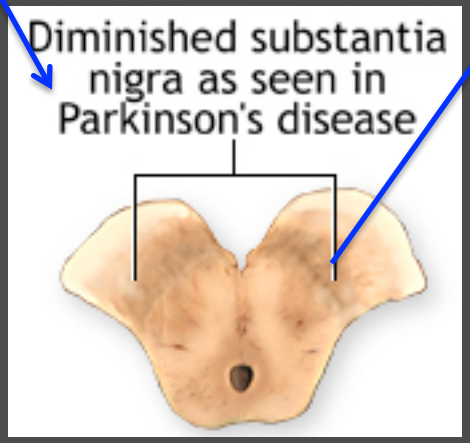
Pathology





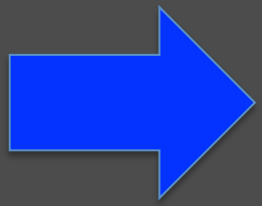


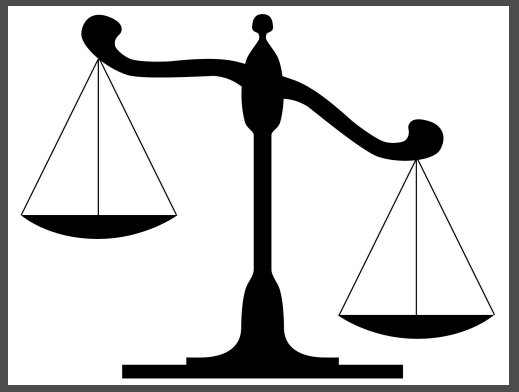
Alpha Synuclein

http://catalog.nucleusinc.com/generateexhibit.php?ID=13231
http://www.nlm.nih.gov/medlineplus/ency/images/ency/fullsize/19515.jpg
http://www.idsia.ch/NNcourse/brain.html

Alpha Syn imbalance

Lewy bodies





Protein Imbalance

What's causing the imbalance?

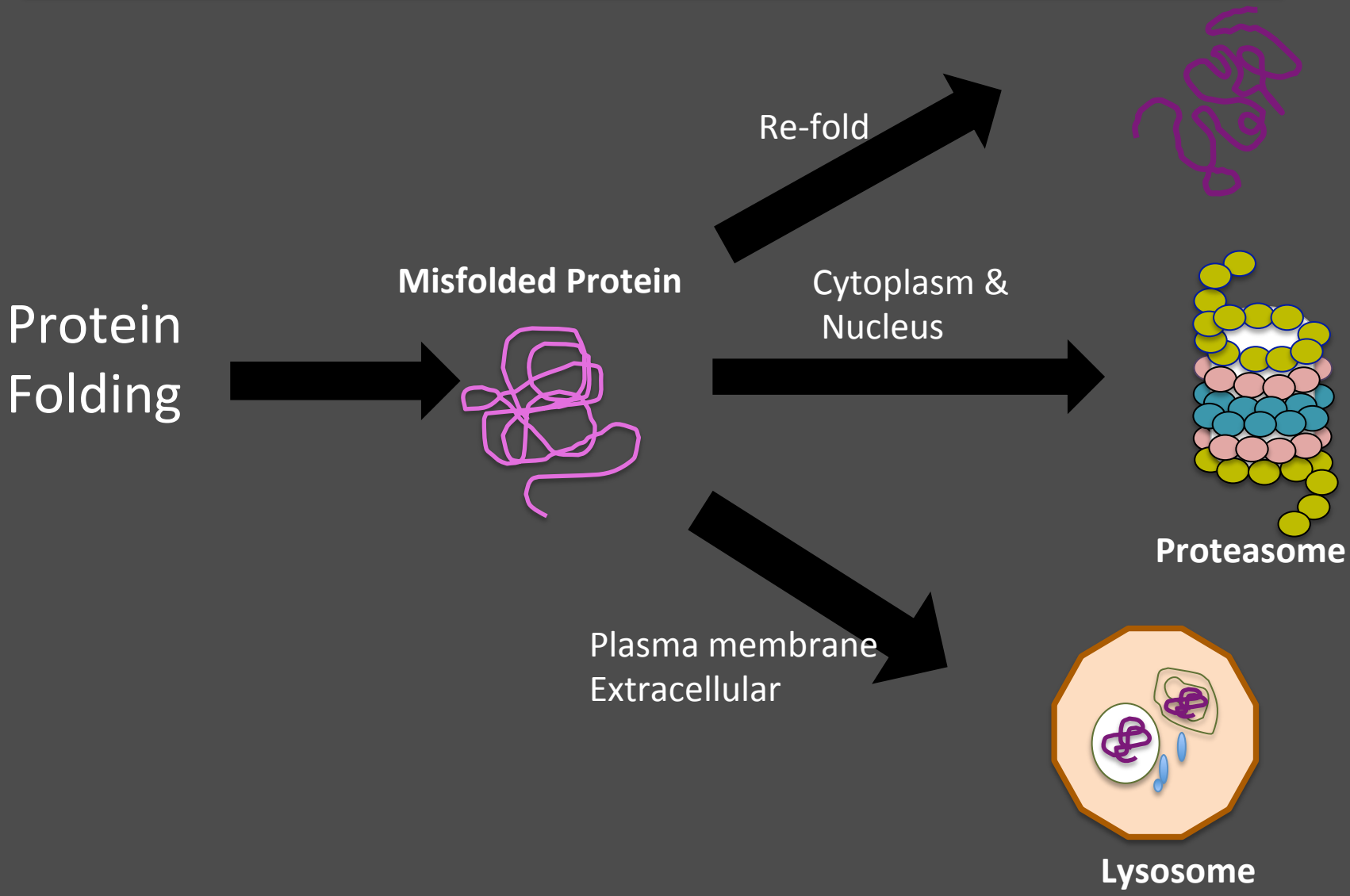
Protein Imbalance

Degrading too little protein?

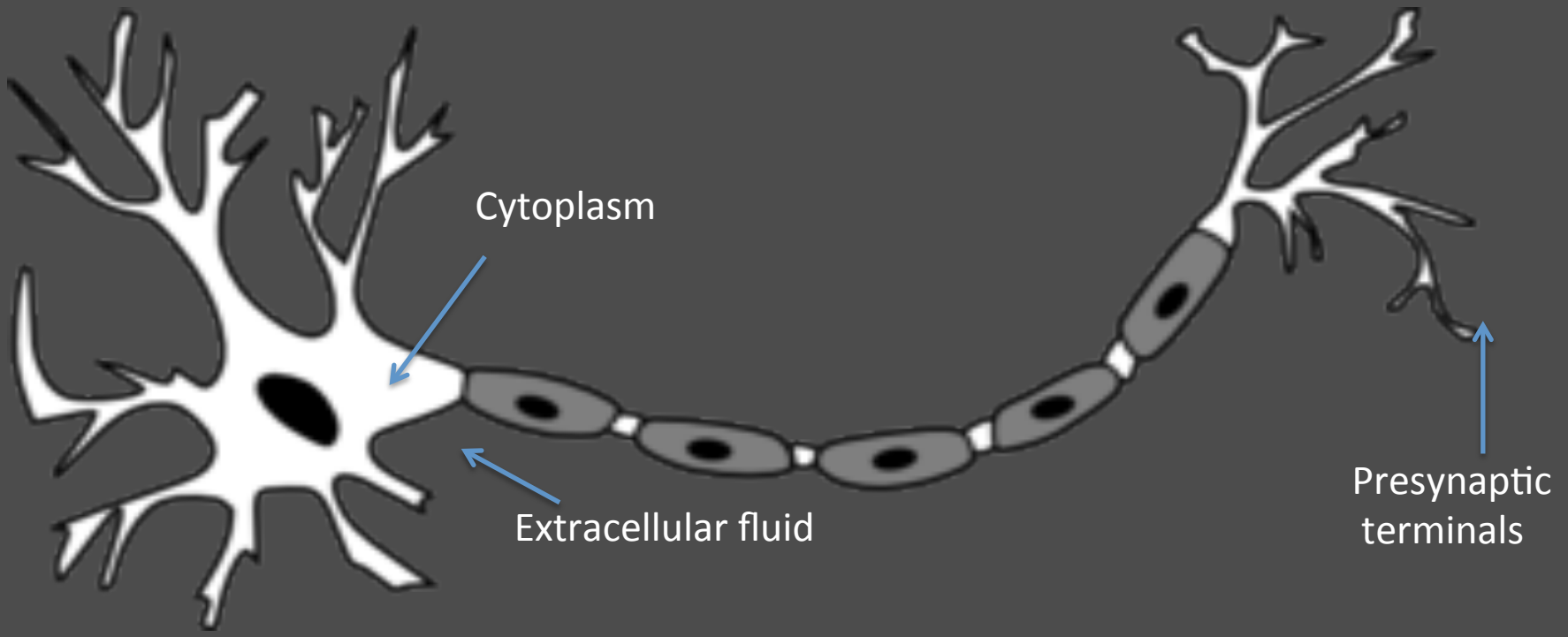
Making too much Protein?

Both?

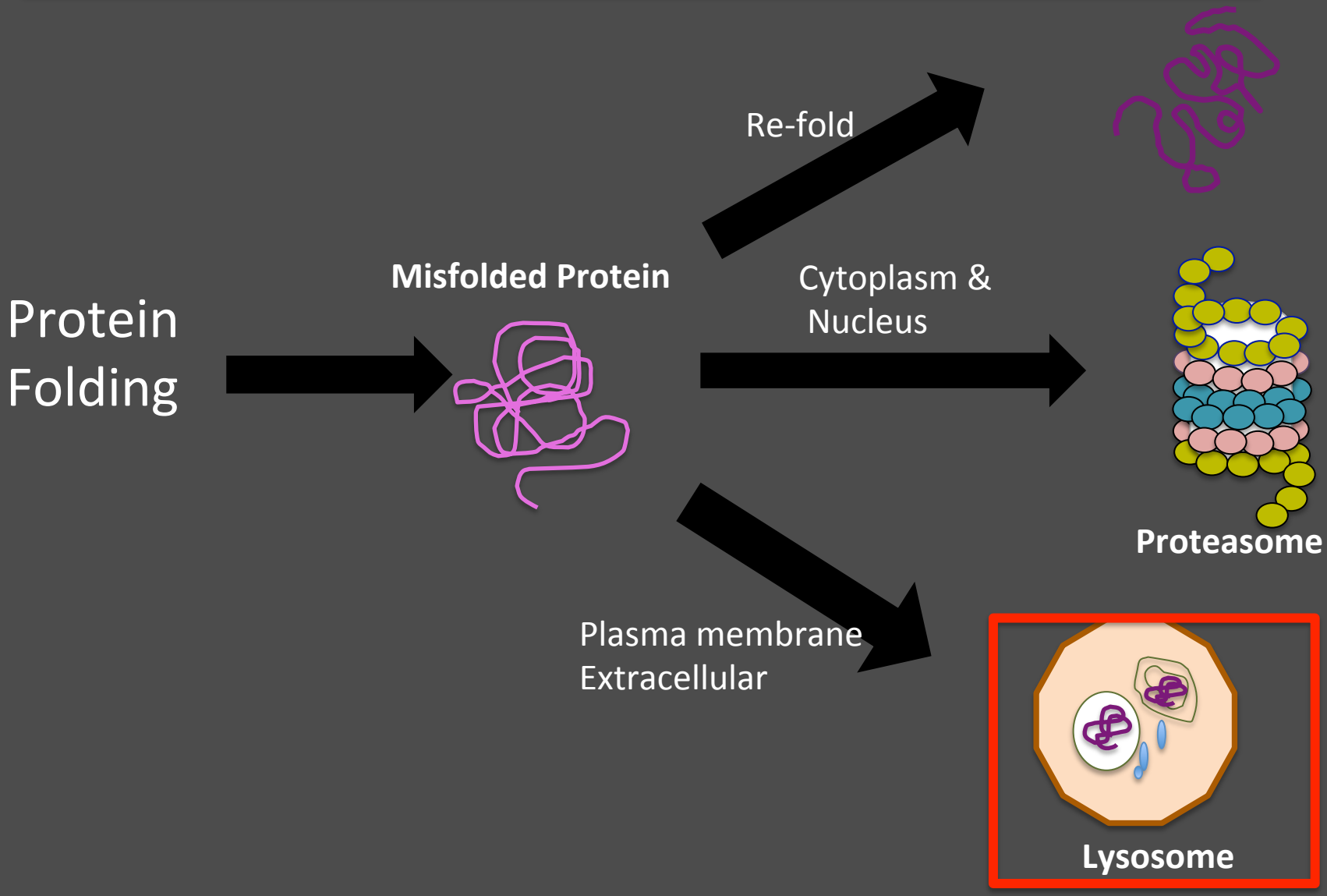
Response to misfold



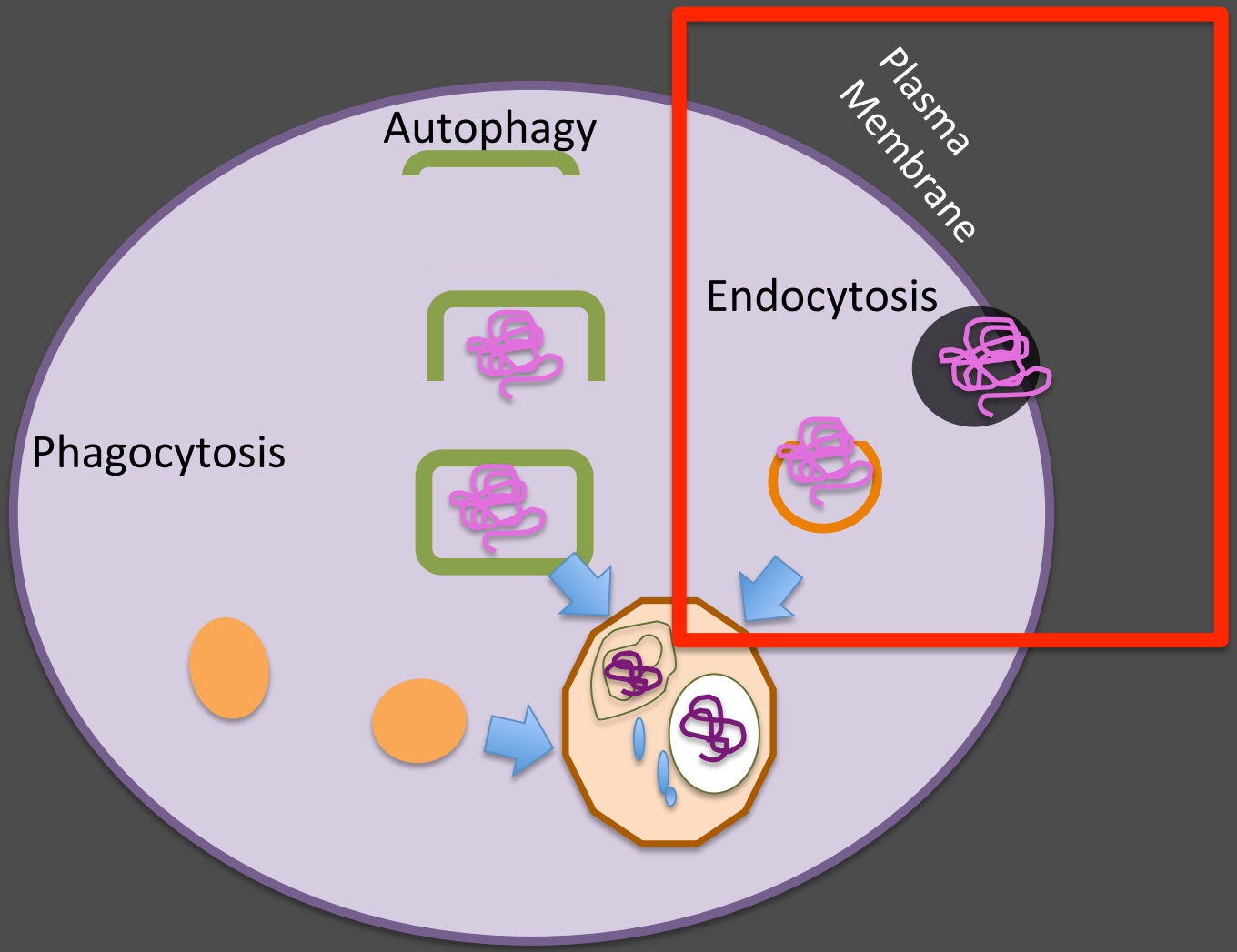
Alpha Synuclein



Response to misfold



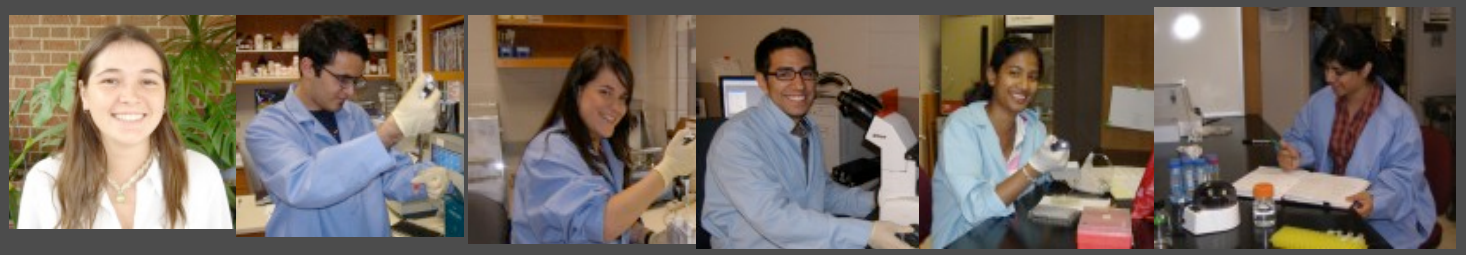
Lysosomal Degradation

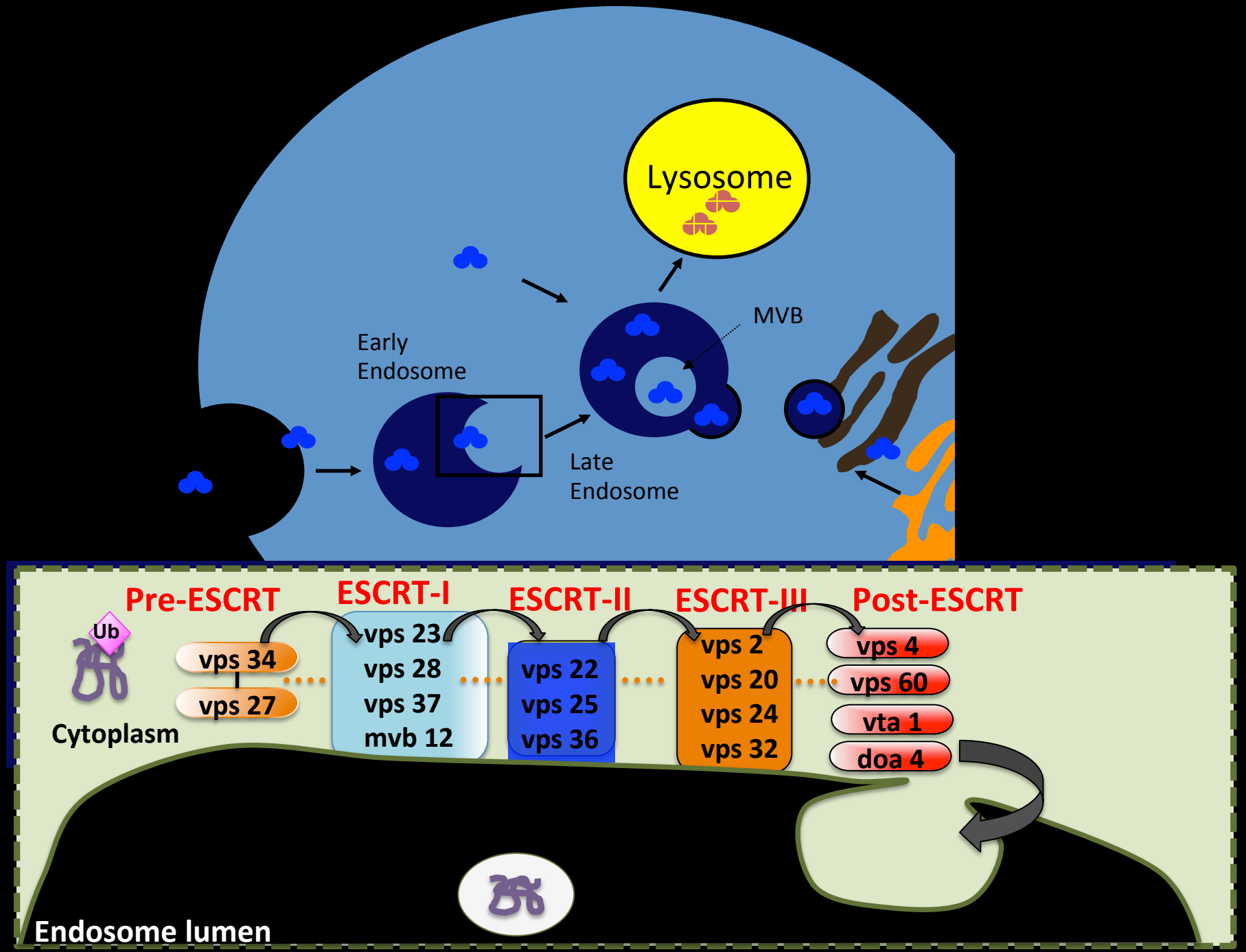


Previous Work

Autophagy –Ray Choi, Alina Konnikova, Daniel Sanchez, Kayla Ahlstrand and Pete Sullivan

Endocytosis- Jessica Price, Mithaq Vahedi, Alexandra Ayala, Jaime Perez and Madhavi Senegolage, Rida Khan

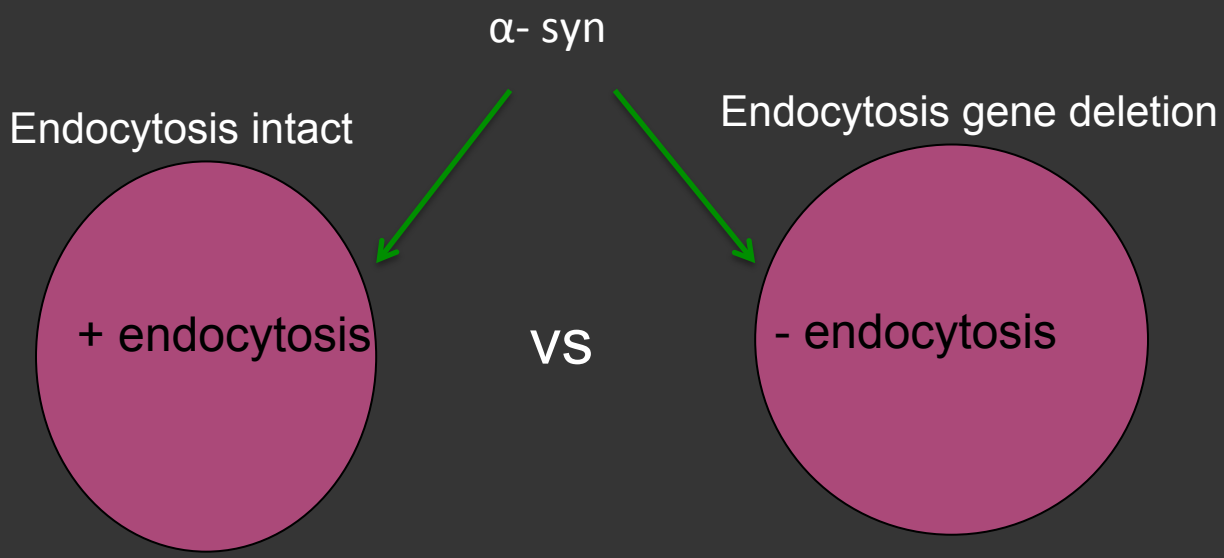



Lysosome
MVB
Early
Endosome
Late
Endosome
Pre-ESCRT
ESCRT-I
ESCRT-II
ESCRT-III
Post-ESCRT
Ub
vps 34
vps 27
vps 23
vps 28
vps 37
mvb 12
vps 22
vps 25
vps 36
vps 2
vps 20
vps 24
vps 32
vps 4
vps 60
vta 1
doa 4
Cytoplasm
Endosome lumen

A Genetic Approach

Compromising Endocytosis

Hypothesis: Impaired endocytosis affects alpha-synuclein pathological properties



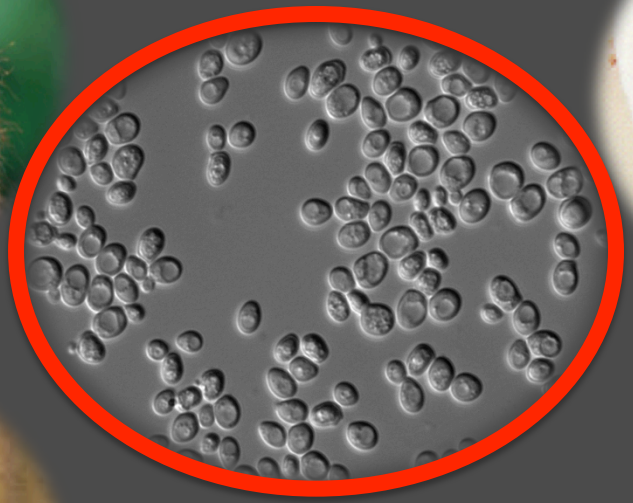
Does α-synuclein localization change?

Does α-synuclein accumulate more?

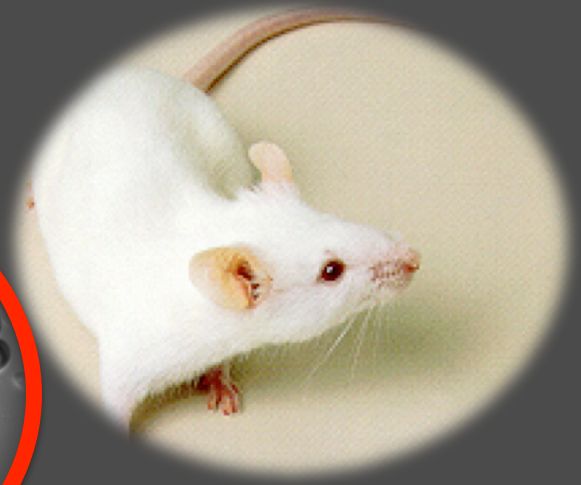
Are more cells toxic?

Model organisms

Primates



Yeast



Mice

Worms

Flies

news.nationalgeographic.com/.../1_461.jpg

http://farm4.static.flickr.com/3186/2993341786_e9412e91dc.jpg
http://news.bbc.co.uk/olmedia/230000/images/_232608_c_elegans_300.jpg
http://www.columbia.edu/itc/cerc/danoff-burg/invasion_bio/inv_spp_summ/white%20mouse.jpg

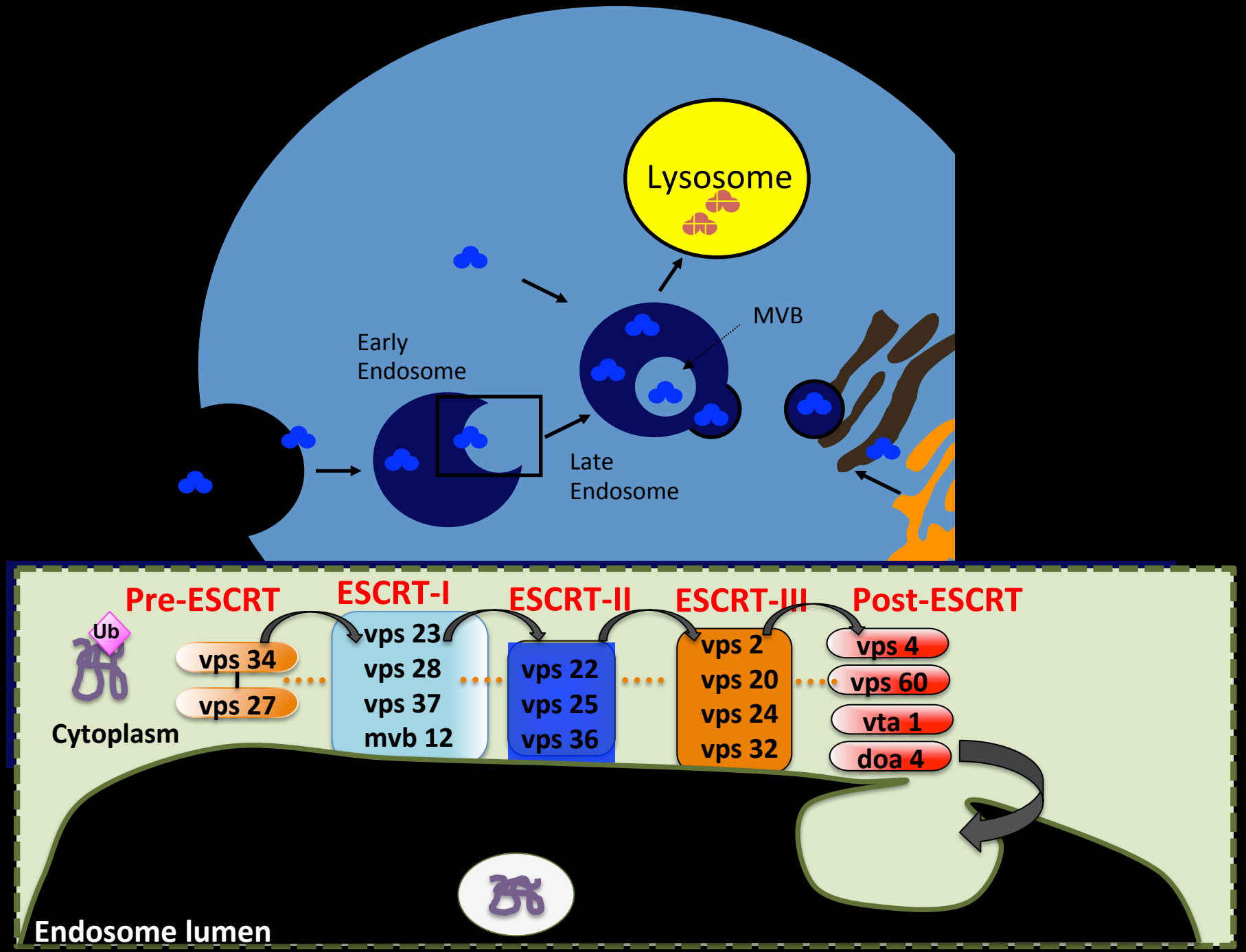
Why Yeast?

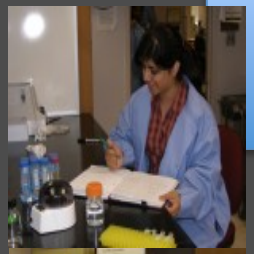
- Inexpensive
- Easy to grow
- Easy to manipulate genes

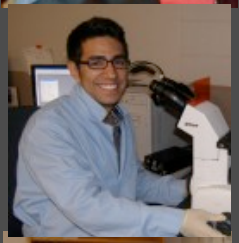
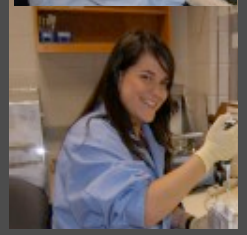
good for undergraduate research

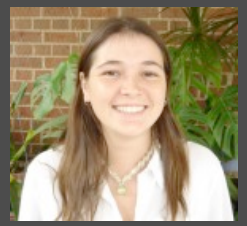
- Make, fold, and degrade proteins just like humans
- Endocytosis is conserved between humans and yeast

Budding Yeast


Lysosome
MVB
Early
Endosome
Late
Endosome
Pre-ESCRT
ESCRT-I
ESCRT-II
ESCRT-III
Post-ESCRT
Ub
vps 34
vps 27
vps 23
vps 28
vps 37
mvb 12
vps 22
vps 25
vps 36
vps 2
vps 20
vps 24
vps 32
vps 4
vps 60
vta 1
doa 4
Cytoplasm
Endosome lumen





Endocytosis Summary

Strains	Change in Localization	Increased Accumulation	Toxicity
Pre-ESCRT			
vps34	Yes	Yes	No
vps27	Yes	None	No
ESCRT-I			
vps23Δ	No	None	No
vps28Δ	Yes	Yes	Yes
vps37Δ	Yes	Yes	No
mvb12Δ	No	Yes	No
ESCRT-II			
vps22Δ	Yes	None	No
vps25Δ	Yes	Yes	No
vps36Δ	Yes	Yes	No
ESCRT-III			
vps2Δ	No	Yes	No
vps20Δ	Yes	Yes	No
vps24Δ	No	None	No
vps32Δ	Yes	None	No
Post-ESCRT			
vps4Δ	Yes	Yes	No
vps60Δ	Yes	Yes	No
doa4Δ	Yes	Yes	No
vta1Δ	Yes	Yes	No

-Localization : 13
-Accumulation: 12
-Toxicity: 1

vps28Δ
Is
Key

New Question

Does synuclein concentration in *vps28Δ* affect this toxicity?

Higher concentration will result in:

- More aggregates, more cytoplasmic
- More accumulation
- More toxicity

Aims -> Predictions

LOW CONC

vps28
WT

VS

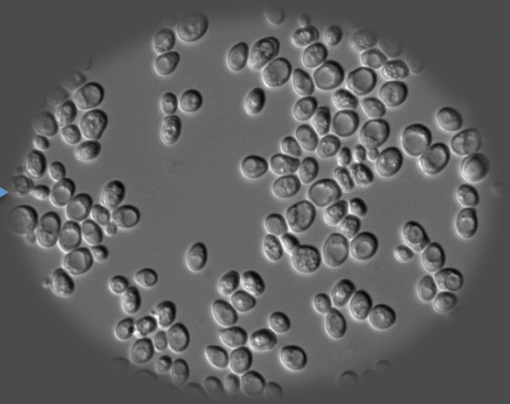
vps28Δ
WT

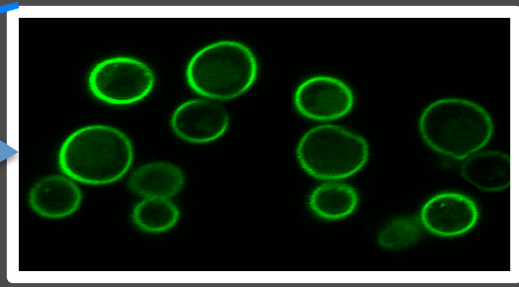
HIGH CONC

vps28
WT

vps28Δ
WT

Question 1: More aggregates?





http://poetryourworlds.blogspot.com/2012/03/green-glow-light-bulb-glowinggreen.html
http://www.clker.com/clipart-blue-light-bulb.html

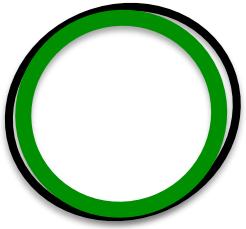
http://biology.unm.edu/ccouncil/Biology_203/Images/Microscopes/microscope6.jpeg

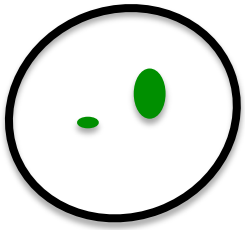
Question 1: More aggregates?

Low Conc

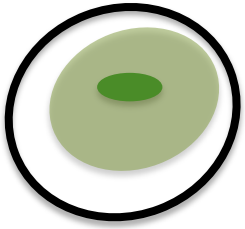
High Conc

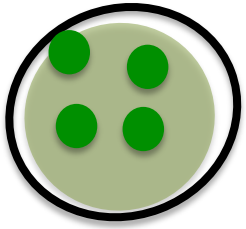
+vps28





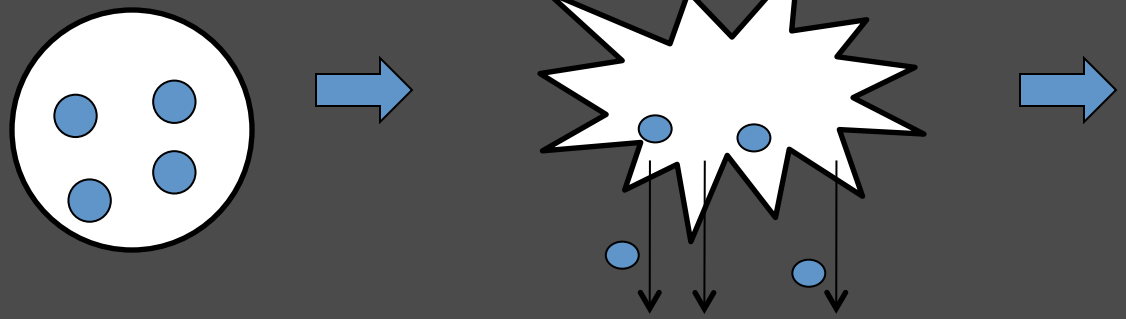
-vps28





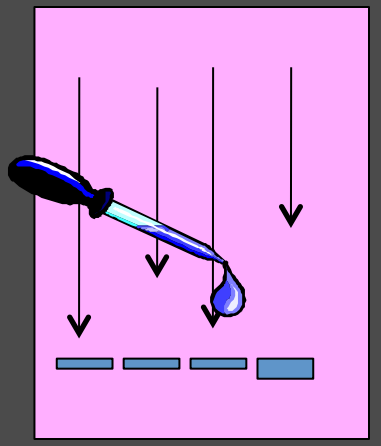
Question 2: More accumulation?

1. Cell makes proteins

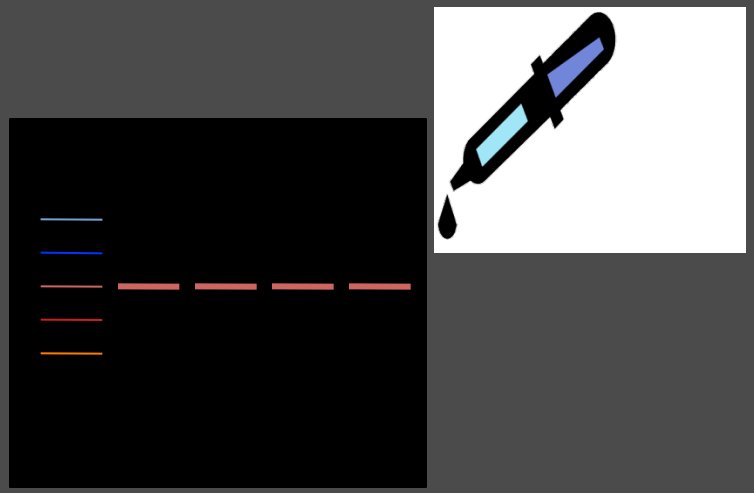


2. Break up cells and obtain proteins

3. Transfer to gel



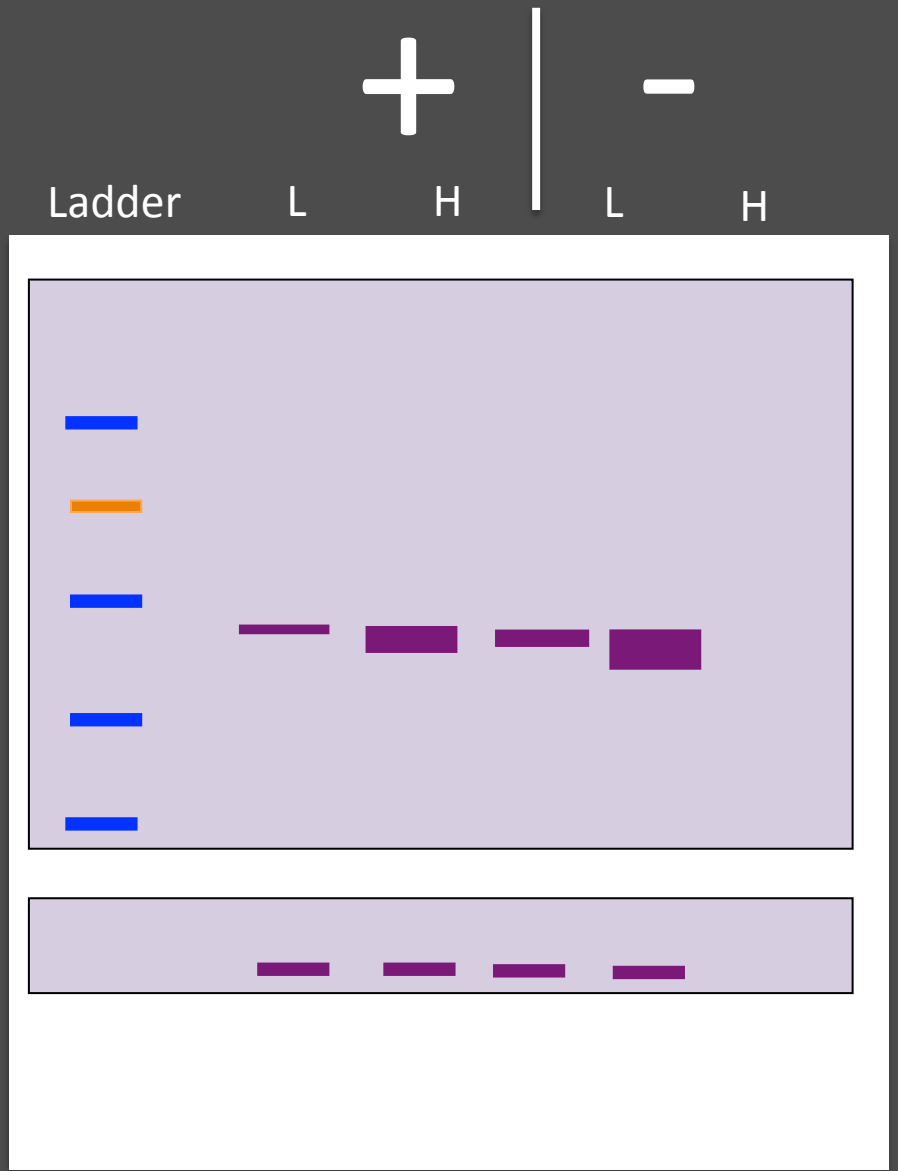
4. Add antibody



Question 2: More accumulation?

vps28

Alpha syn Concentration



Alpha Syn

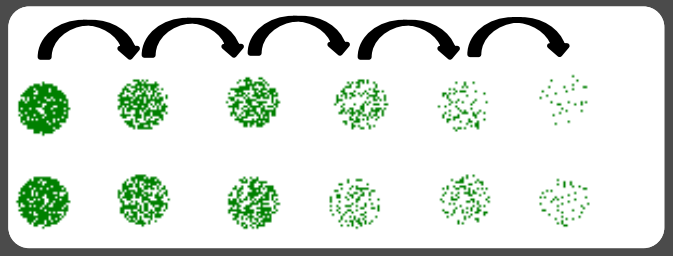
PGK

Question 3: More toxicity?

TEST: SPOTTING

Control

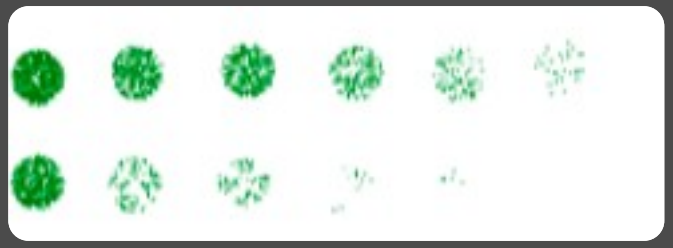
Test



No Toxicity

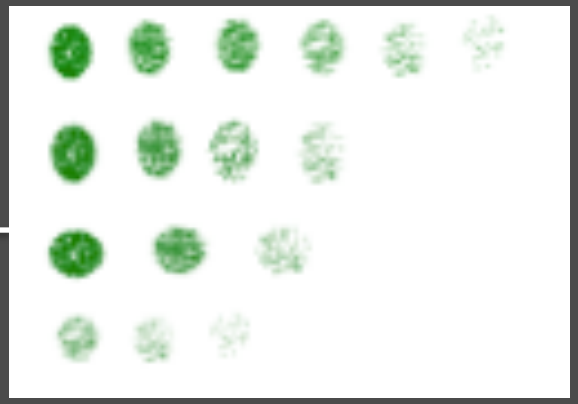
Control

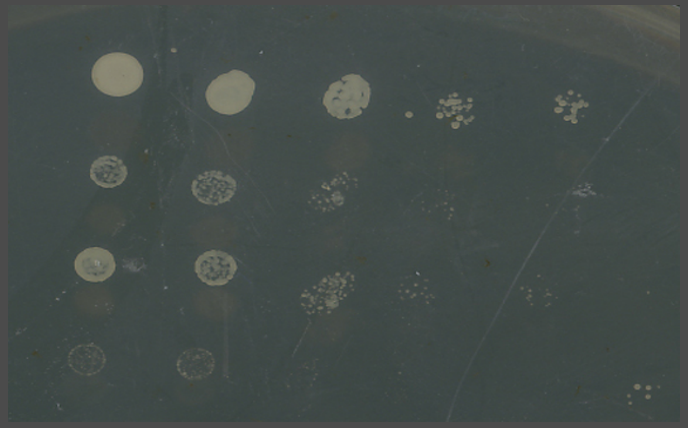
Test



Toxicity

Question 3: More toxicity?

	Alpha Syn	
vps28 +	L	
	H	
−	L	
	H	



YES!

Conclusion

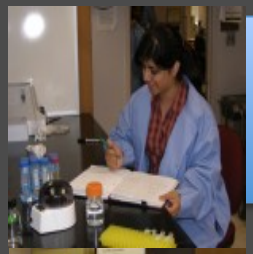
Alpha synuclein concentration ↑ Toxicity ↑

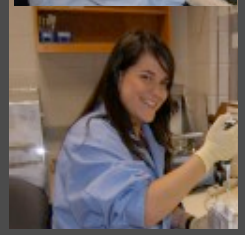
- More cytoplasmic
- More accumulated More Toxic

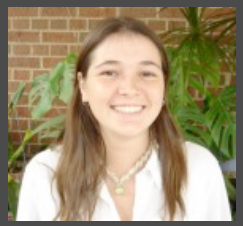
✔

Future Studies:

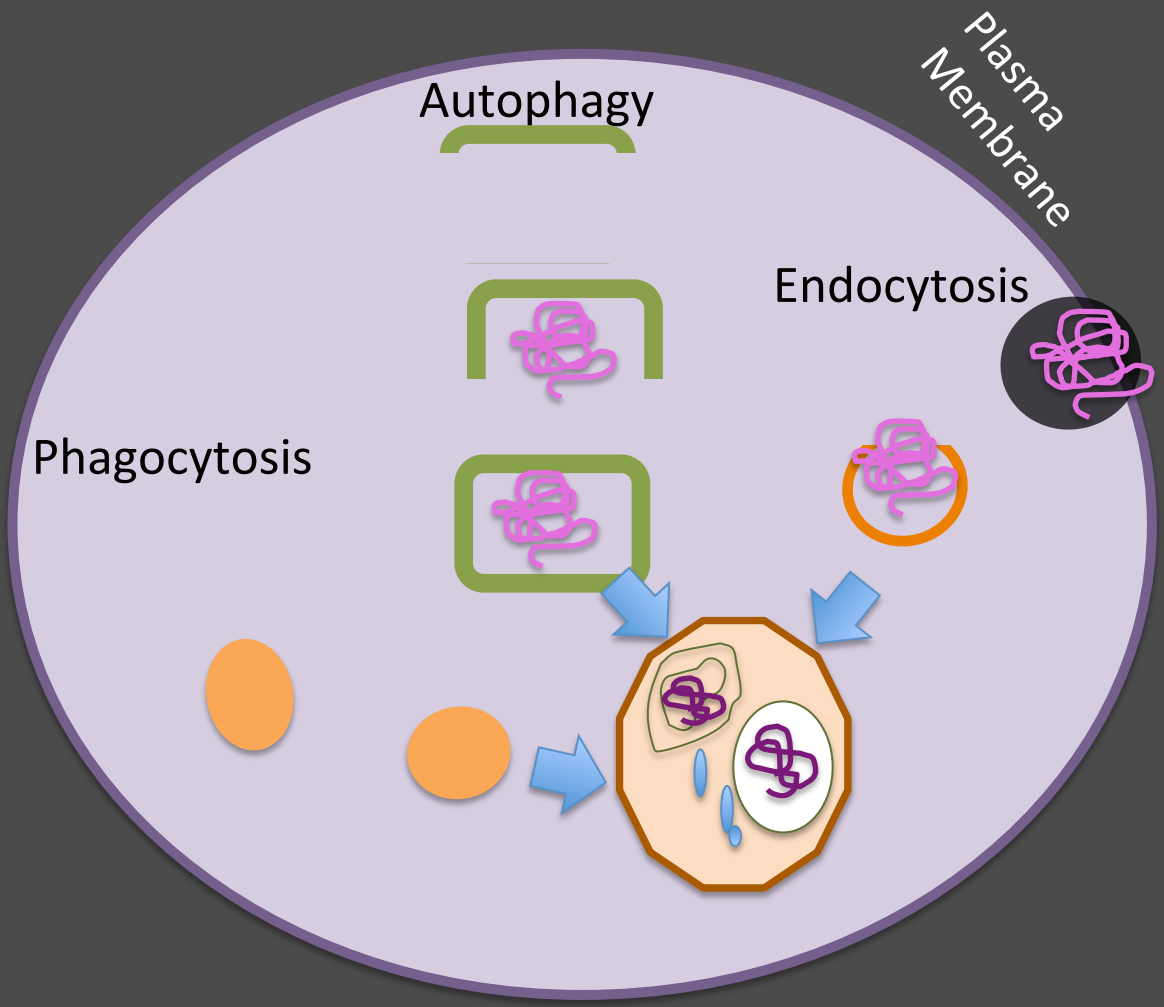
- Fission Yeast
- More repeats



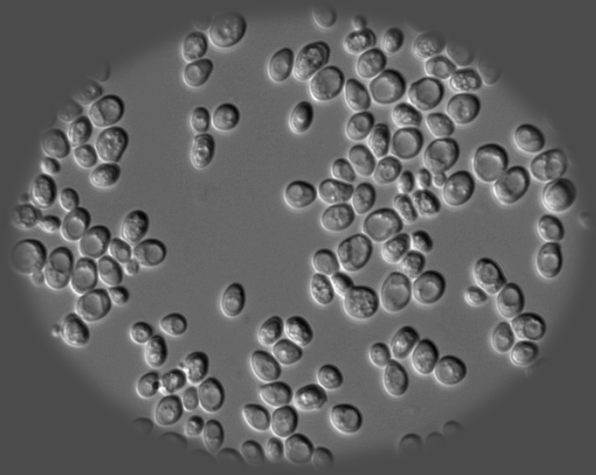


Importance



Importance

+



+ Undergrad

http://www.google.com/imgres?q=Parkinsons+Disease+patient&hl=en&biw=1096&bih=628&tbm=isch&tbnid=Mas2sJesSWTY0M:&imgrefurl=http://www.diyhealth.com/new-evidences-bolster-pesticides-parkinsons-disease-links.html&docid=dMh3_-SdUzy1lM&imgurl=http://www.instablogsimages.com/images/2007/04/24/parkinsons-disease-patient_9.jpg&w=250&h=248&ei=XiCIUJycF-eS2AX4koDQBg&zoom=1&iact=rc&dur=2&sig=112468104117006570770&page=2&tbnh=135&tbnw=149&start=17&ndsp=20&ved=1t:429,r:10,s:20,i:162&tx=75&ty=66

Acknowledgements

I would like to thank:

•Dr. DebBurman

•The DebBurman lab
- Madhavi Senagolage
- Natalie Kukulka
- Katrina Campbell
- Galina Lipkin
- Michael Herriges